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Linkage of Familial Dilated Cardiomyopathy with Conduction Defect and Muscular Dystrophy to Chromosome 6q23

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Summary

Inherited cardiomyopathies may arise from mutations in genes that are normally expressed in both heart and skeletal muscle and therefore may be accompanied by skeletal muscle weakness. Phenotypically, patients with familial dilated cardiomyopathy (FDC) show enlargement of all four chambers of the heart and develop symptoms of congestive heart failure. Inherited cardiomyopathies may also be accompanied by cardiac conduction-system defects that affect the atrioventricular node, resulting in bradycardia. Several different chromosomal regions have been linked with the development of autosomal dominant FDC, but the gene defects in these disorders remain unknown. We now characterize an autosomal dominant disorder involving dilated cardiomyopathy, cardiac conduction-system disease, and adult-onset limb-girdle muscular dystrophy (FDC, conduction disease, and myopathy [FDC-CDM]). Genetic linkage was used to exclude regions of the genome known to be linked to dilated cardiomyopathy and muscular dystrophy phenotypes and to confirm genetic heterogeneity of these disorders. A genomewide scan identified a region on the long arm of chromosome 6 that is significantly associated with the presence of myopathy (D6S262; maximum LOD score [Z_{max}] 4.99 at maximum recombination fraction [θ_{max}] .00), identifying FDC-CDM as a genetically distinct disease. Haplotype analysis refined the interval containing the genetic defect, to a 3-cM interval between D6S1705 and D6S1656. This haplotype analysis excludes a number of striated muscle–expressed genes present in this region, including laminin α2, laminin α4, triadin, and phospholamban.

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Introduction

Dilated cardiomyopathy has a prevalence of 36/100,000. It is a significant cause of congestive heart failure (CHF) and the major indication for orthotopic cardiac transplantation (Codd et al. 1989; Dec and Fuster 1994; Kelly and Strauss 1994). The etiology of dilated cardiomyopathy is multifold and includes ischemic, immunological, toxic, and genetic causes. Echocardiographic studies of asymptomatic first-degree relatives of individuals with idiopathic dilated cardiomyopathy estimate that 20%–30% of idiopathic dilated cardiomyopathy is familial (Michels et al. 1992). Recent genetic linkage studies have implicated several different chromosomal loci with the development of four-chamber cardiac dilation and CHF, confirming the genetic heterogeneity of familial dilated cardiomyopathy (FDC) (Kass et al. 1994; Durand et al. 1995; Krajinovic et al. 1995; Olson and Keating 1996; Bowles et al. 1996). One difficulty in identifying defective genes in FDC has been the lack of large families with affected, living members and sufficient power for genetic linkage studies. Despite this, five different genetic loci have been linked to the pathogenesis of autosomal dominant FDC, but the defective genes remain unknown (table 1).

Of the five loci implicated in FDC, two are associated with phenotypes that include cardiac conduction defects in addition to dilated cardiomyopathy. The phenotypes linked to 1p11-1q11 and 3p22-25 initially manifest with sinoatrial (SA) or atrioventricular (AV) block, resulting in slow heart rates and syncope (Graber et al. 1986; Greenlee et al. 1986; Kass et al. 1994; Olson and Keating 1996). A subset of the affected individuals develops four-chamber cardiac dilation and clinical CHF. These disorders have been designated "FDC-CD1" and "FDC-CD2," respectively, to distinguish their phenotypes from three other disorders that involve FDC without conduction-system disease (isolated FDC). Three chromosomal loci have been linked to isolated FDC: chromosomes 1q32, 9q13, and 10q22-24 (Durand et al. 1995; Krajinovic et al. 1995; Bowles et al. 1996). The penetrance of these disorders is variable and increases with increasing age.

Table 1

Genetic Loci Implicated in Autosomal Dominant FDC

Disorder	OMIM Number	Markers for Z_{max}	Locus[a]	Reference
FDC-CD1	115200	D1S305, D1S303	1p11-1q11	Kass et al. (1994)
FDC-CD2	601154	D3S1263, D3S2303	3p22-25	Olson and Keating (1996)
FDC-CDM		D6S262, D6S1040	6q22-23	Present report
FDC	601493	D10S607, D10S201	10q21-23	Bowles et al. (1996)
FDC	601494	D1S414, D1S505	1p32	Durand et al. (1995)
FDC	600884	D9S153, D9S152	9q12-13	Krajinovic et al. (1995)

[a] Six autosomal regions that have been associated with inherited dilated cardiomyopathy; the first three are linked to dilated cardiomyopathy and conduction-system disease affecting the SA and/or AV nodes, whereas the second three are linked to isolated dilated cardiomyopathy.

Mutations that cause cardiac dysfunction may also result in skeletal muscle weakness owing to the expression of the defective gene in all types of striated muscle. Myocyte hypertrophy, myocyte loss, and progressive replacement by connective tissue are common histopathological features seen in both dilated-cardiomyopathy hearts and dystrophic muscle. However, not all of the myopathic syndromes affect both cardiac and skeletal muscle. For example, limb-girdle muscular dystrophy (LGMD) type 1A, a dominant disorder that links to the long arm of chromosome 5, appears to affect only skeletal muscle (Speer et al. 1992). In contrast, the recently described LGMD type 1B involves an adult-onset, proximal, progressive muscular dystrophy and a cardiac phenotype that involves conduction-system disease affecting the AV node and, in some patients, four-chamber cardiac dilation with CHF (van der Kooi et al. 1996). LGMD type 1B recently has been linked to the same microsatellite markers as FDC-CD1 (Kass et al. 1994; van der Kooi et al. 1997). The segregation of LGMD type 1B and FDC-CD1 with the same markers at 1q11-1p11 may indicate that these disorders are allelic. The genes that influence the development of cardiac versus skeletal muscle disease are not known, although it is clear from analysis of dystrophin, the defective gene in Duchenne muscular dystrophy and X-linked FDC, that mutations in a single gene may result in skeletal muscle weakness, CHF, bradyarrhythmias, or varying combinations of these symptoms (Berko and Swift 1987; Muntoni et al. 1993; Towbin et al. 1993).

In this report, we describe a disorder that includes autosomal dominant dilated cardiomyopathy, cardiac conduction-system disease, and an adult-onset, slowly progressive muscular dystrophy that involves the proximal musculature (FDC-CDM). Younger affected individuals who lack skeletal muscle symptoms may show conduction defects that include right-bundle-branch block (RBBB) or first-degree AV block. As the disease progresses, high-grade AV block appears accompanied by cardiac dilation that is not hypertrophic. CHF and symptoms of muscle weakness tend to occur later in the disorder. Genetic linkage analysis was performed and excluded regions already implicated in dilated cardiomyopathy and muscular dystrophy. A genomewide linkage approach was used to identify the locus responsible for this disorder, and significant linkage was found at 6q23 (D6S262; Z_{max} 4.99 at $\theta_{max} = .00$). A number of candidate genes near the region—laminin $\alpha 2$, laminin $\alpha 4$, phospholamban, and triadin—are excluded by recombination events between these genes and FDC-CDM. Haplotype analysis has refined the interval containing the FDC-CDM gene to 3 cM between D6S1705 and D6S1656.

Subjects and Methods

Clinical Evaluations

Clinical history was ascertained on living members through interviews, examinations, and medical records. The affectation status of deceased members was determined from medical records or reports from other family members. Informed consent was obtained under the approval of institutional review boards (The Children's Hospital, The University of Chicago Hospitals, and Duke University Medical Center), and blood was drawn for DNA analysis. Affectation status was determined by the presence of any of the following: (1) abnormal electrocardiography (EKG) showing RBBB or second- or third-degree heart block, (2) echocardiography showing dilated cardiomyopathy with elevated end-systolic and end-diastolic diameters and/or reduced ejection fraction, (3) muscular dystrophy with proximal muscle weakness, (4) abnormal muscle biopsy with variable fiber size and increased connective tissue, or (5) creatine kinase elevated more than two times normal control values. Unaffected individuals whose genotyping data were used in LOD-score calculations were >25 years of age and had been evaluated by a cardiologist and neurologist.

Genotyping

Genomic DNA was purified by use of PureGene (Gentra Systems) (Miller et al. 1988). Genotyping was performed with an Applied Biosystems 377 automated DNA sequencer using 4.25% acrylamide (SeaQuate DNA sequencing solution; Sooner Scientific). For genomewide linkage, the Weber 8 microsatellite repeat markers (Research Genetics) were used. Reactions mixtures contained 30 ng of genomic DNA in a 10-μl volume of 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM each dNTP, and 0.5 units of AmpliTaq Gold (Perkin-Elmer Applied Biosystems). Amplifications were performed in an MJ Research PTC-100-96 thermocycler at 94°C for 10 min, followed by 36 cycles of 55°C for 1 min, 72°C for 30 s, and 94°C for 25 s, followed by a single extension at 72°C for 10 min. Typically, four or five PCR primer pairs were combined per PCR reaction vessel, with a final concentration of 0.08 mM of each primer. For electrophoretic multiplexing, an average of 10–12 markers were combined per lane, according to the recommendations of Research Genetics. Allele sizes were calculated on the basis of an internal size standard in each lane (Genescan-350 TAMRA; Perkin-Elmer Applied Biosystems). Allele sizes were analyzed by use of Genescan and Genotyper software (Perkin-Elmer Applied Biosystems) on a Macintosh 7600. Markers not part of the Weber 8 set were obtained from Research Genetics and were labeled with R110-dCTP (Perkin-Elmer Applied Biosystems) at a ratio of 1:700 (labeled:unlabeled dCTP). Approximately 1/10th of the PCR product was loaded onto the gels for analysis.

Linkage Analysis

All genotypes were scored blinded to phenotype. Allele information was entered into Cyrillic (Cherwell Scientific) and was downloaded for MLINK and ILINK analysis in FASTLINK 3.0P based on LINKAGE 5.1 (Lathrop et al. 1984). Two-point linkage analysis was performed by use of the LINKAGE 5.1 package, on SoftWindows 2.0 (Insignia Solutions) on a Power Macintosh 7600 or a Sun Ultra 1. Penetrance was set at 1, and the disease-allele frequency was assumed to be .000005. This allele frequency was used because there are at least six different loci associated with dilated cardiomyopathy. Furthermore, the combination of FDC and muscular dystrophy is more rare than dilated cardiomyopathy alone. Allele frequencies were set at equal, $1/n$ frequencies, where n is the number of alleles at the marker locus. After the identification of linkage, estimates of penetrance and allele frequencies were varied to assess the robustness of the conclusions. In addition to these analyses, "affecteds-only" or low-penetrance analyses were performed. This conservative analysis utilizes marker-genotype data on all family members while retaining the phenotypic data on affected family members only. Thus, the contribution of the more certain phenotypic data to the overall LOD score is evaluated. Multipoint analysis was performed with the FASTLINK version of LINKMAP. Marker order and intermarker distances were based on existing linkage maps (Genome Database 1996 [Généthon March 1996, SGC 1996, or Whitehead Contig WC6.15 November 1996 maps]). For multipoint analysis, the number of alleles was reduced without loss of information, and the allele frequencies were set at $1/n$.

Results

Clinical Findings

A summary of the symptoms of the affected members of the kindred is shown in table 2. History was obtained on 60 members of the pedigree, and 25 were identified as affected, on the basis of the presence of cardiac conduction defects, cardiomyopathy, and/or muscle weakness (fig. 1). At age 37 years, the proband (IV-29) underwent evaluation for cardiac transplantation. He reported symptoms of muscle weakness as early as age 17 years. Symptoms of dyspnea on exertion had been present since age 25 years. A pacemaker had been implanted at age 29 years, because of syncopal episodes due to complete heart block. Echocardiography revealed increased end-systolic and end-diastolic diameters, 4.3 cm and 5.5 cm, respectively, with an estimated ejection fraction of 35%. Biatrial enlargement was also noted. Ventricular-wall thickness was not increased. At cardiac catheterization, the pulmonary-capillary-wedge pressure was found to be elevated at 20 mm Hg; no evidence of coronary artery disease was seen on angiography. The proband suffered ventricular tachycardia and died while awaiting cardiac tranplantation. The proband's father died at age 49 years, of CHF and arrhythmia. Two of the proband's siblings, IV-28 and IV-30, were evaluated by cardiac echocardiography at ages 34 and 32 years, respectively, and were found to have preserved systolic function, but they complained of mild dyspnea on exertion. IV-28 had a 3–5-year history of mild muscle weakness and had had a pacemaker implanted for complete heart block. Ventricular tachycardia was noted on Holter monitoring. IV-30 had episodes of paroxysmal atrial fibrillation, elevated serum creatine phosphokinase (CPK), and an EKG showing diffuse ST-T–wave abnormalities and preserved intervals. Cardiac catherization showed normal coronary arteries. At age 27 years, IV-31 complained of mild dyspnea on exertion and had RBBB on EKG. At age 23 years, IV-32 denied muscle weakness or dyspnea on exertion.

Table 2

Symptoms in FDC-CDM

Patient	DCM[a]	Pacemaker	Arrhythmia[b]	CHF Symptom(s)[c]	Muscle Symptom(s)[d]	Muscle Biopsy[e]	Age at Death (years)
I-1				Yes	Weakness		54
II-2				Yes			Unknown
II-4				Yes			39
II-8	$(+)^{X}$		2:1 HB	Yes			57
I-12							Unknown
II-16				Yes	Weakness		63
III-3	$(+)^{X,E,P}$	+	HB	II–III	Weakness	D	68
III-4	$(+)^{X,E,P}$	+	HB	II–III	Weakness	D	Living
III-7				Yes	None		29
III-11	$(+)^{X,E,P}$		HB-2	I–II	Weakness	D	Living
III-13	$(+)^{X,E,P}$			I–II	Weakness	D	Living
III-15	$(+)^{X,E,P}$	+	HB-3	I–II	Weakness	D	Living
III-20				Yes	Weakness		Unknown
III-23					Weakness		Living
III-27	$(+)^{X,P}$	+	HB-3 Sudden death	Yes	Weakness	D	50
IV-2		+	HB		Weakness		Living
IV-3		+	HB		Weakness		Living
IV-5		+	HB				Living
IV-12					Weakness		Living
IV-15					Increased CPK, calf hypertrophy		Living
IV-24			Sudden death		None		25
IV-28	$(-)^{X,E,P}$	+	HB-3, VT	I–II	None		Living
IV-29	$(+)^{X,E,P}$	+	HB-3, VT Sudden death	III–IV	Weakness	D	37
IV-30	$(-)^{X,E,P}$		PAF	I–II	Increased CPK	D	Living
IV-31			RBBB	I–II	None		Living

[a] Superscript letters indicate method of ascertainment: X = x-ray; E = 2D electrocardiographic evaluation; and P = physical examination. Diagnostic criterion/criteria for dilated cardiomyopathy were echocardiography showing increased end-systolic, increased end-diastolic, or reduced ejection fraction; x-ray showing enlarged heart; and/or physical examination showing laterally displaced point of maximum impulse.

[b] HB = heart block by history (details unavailable); HB-2 = second-degree heart block; HB-3 = third-degree heart block; VT = ventricular tachycardia; PAF = paroxysmal atrial fibrillation; and RBBB = right bundle branch block.

[c] By New York Heart Association heart-failure classification, when available.

[d] Weakness = weakness in a limb-girdle distribution; and Increased CPK = more than two to four times normal control levels. All patients remained ambulatory, except for IV-29, who remained so until immediately prior to his death.

[e] D = dystrophic changes apparent on light microscopy.

The clinical phenotype encompassed a spectrum of severity that increased with increasing age. The youngest affected individuals had serum CPK that was elevated to two to four times that in a normal control, first-degree heart block, or RBBB. Elevation of serum CPK was variably present and was usually two to four times that in a normal control. Serum CPK elevation was more likely to be present in males than in females but was not reliable as a diagnostic measure, since at least one individual with muscle weakness (IV-12) did not have elevated serum CPK. Three individuals had sudden death; one was without symptoms of either CHF or muscle weakness at the time of his death. Four-chamber cardiac enlargement, with no increase in wall thickness, accompanied by mitral, aortic, and tricuspid regurgitation, developed in the later phases of the illness. Symptoms of CHF varied in age at onset. In the late 3d decade, the proband, IV-29, and his siblings, IV-28 and IV-30, developed symptoms consistent with the New York Heart Association classification of heart failure class I–II. In contrast, III-11, III-13 and III-15 did not develop symptoms of dyspnea on exertion until the 4th or 5th decade. Muscle weakness was present in a proximal distribution and progressed in a pattern similar to that of cardiac involvement. Facial muscles appeared spared. All affected individuals remained ambulatory throughout their lifetime. Muscle biopsies, when available, showed dystrophic architecture with variation in fiber size, centrally placed

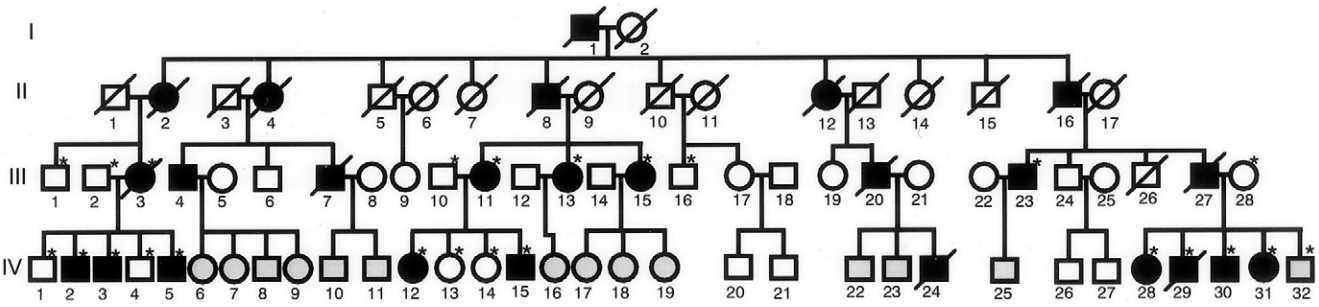

Figure 1 FDC-CDM pedigree used for linkage analysis. A single large pedigree with FDC-CDM was ascertained. Affected members of the pedigree are denoted by blackened symbols; unaffected members are denoted by unblackened/unshaded symbols; members of the pedigree who were not of sufficient age to allow determination of their affectation status are denoted by gray-shaded symbols; and asterisks (*) indicate members on whom DNA analysis was performed. The pedigree is five generations; the fifth generation is not of sufficient age and has been excluded. Autosomal dominant inheritance was assumed. No decrease in the age at onset was seen in generations in II–IV, arguing against genetic anticipation.

nuclei, and increased connective tissue and fat. Cardiac biopsies were unavailable.

Twenty individuals were identified as unaffected, and 15 were scored as "phenotype unknown," given their age (<25 years). All affected members of the pedigree developed symptoms at age <25 years, but few had symptoms at age <20 years. The majority of the fifth generation (not shown) was not of sufficient age to allow determination of their affectation status and was not included in LOD-score calculations. The inheritance pattern of FDC-CDM was consistent with autosomal dominant transmission. Father-to-son transmission excluded the X chromosome. The age at onset was similar in all generations in the pedigree, arguing against genetic anticipation. The family was of French Canadian descent but has been in the United States for four generations. No evidence of consanguinity was present.

Linkage Analysis

The loci previously implicated in dilated cardiomyopathy and muscular dystrophy were excluded as the cause of the genetic defect in FDC-CDM, by LOD-score analyses showing significantly negative results (data not shown). These regions included markers within and surrounding 1p11, 1q32, 3p22-25, 9q13, 10q21-23, 5q22-31, 15q15, 2p13, 13q12, 17q12, 4q12, and 19q13. A genomewide scan to identify the locus responsible for FDC-CDM was initiated with use of markers with high heterozygosity that were 90% tri- and tetranucleotide repeats and that were spaced at an average of 9 cM (Sheffield et al. 1995). These markers offered more accurate allele typing and improved the reliability of the data. The markers were multiplexed both for PCR and for electrophoresis, to improve throughput and reduce the time necessary to identify linkage. Sixty markers, excluding ~35% of the genome, were analyzed when a significant LOD score was found for D6S1040 (Z_{max} 4.34 at θ_{max} = .00; table 3). The maximal two-point LOD score was obtained with D6S262 (Z_{max} 4.99 at θ_{max} = .00). Varying the penetrance to as low as .6 still produced maximal two-point LOD scores >4.00, for both D6S262 and D6S1040. Given the remote French ancestry of the FDC-CDM pedigree, CEPH allele frequencies were substituted for those of D6S262 and D6S457, resulting in negligibly higher two-point LOD scores (data not shown). Two-point LOD scores calculated by use of the affected-only analysis yielded Z_{max} 3.36 and 3.08 at θ_{max} = .00, for D6S262 and D6S1040, respectively.

Multipoint analysis was performed with D6S1040, D6S262, and D6S457, with intervals set at 0.7 cM. A maximum multipoint LOD score of 6.06 was obtained, and the interval from D6S1040 to D6S262 was just as likely for placement of the disease locus as was the interval from D6S262 to D6S457. Haplotype analysis showed that all affected members of the pedigree carry the same-size allele for D6S1040, D6S262, and D6S457 (fig. 2). IV-32 carries the affected haplotype yet has not manifested disease, presumably because of his age (23 years) at examination for this study. Because of phenotypic uncertainty, his genotype data set was not included in the LOD-score calculations. Figure 2 shows that five affected members (III-11, III-13, III-15, IV-12, and IV-15) most likely reflect a single recombination event, occurring during transmission between I-1 to II-8, that involves D6S1705 and markers centromeric to D6S1705. Similarly, D6S1656 and markers telomeric to it show a likely single recombination event having occurred during transmission from I-1 to II-2 or from II-2 to III-3. Four affected members (III-3, IV-2, IV-3, and IV-5) reflect this recombination event. These recombination events define the FDC-CDM interval as being

Table 3

Pairwise LOD Scores Reflecting Linkage for FDC-CDM and Markers of Chromosome 6q23

	LOD Score at Recombination Fraction of								
Locus	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D6S1705	−∞	−1.01	−.43	−.29	−.32	−.38	−.26	...	...
D6S1040	4.34	4.29	3.94	3.49	2.55	1.57	.65	4.34	0
D6S262	4.99	4.90	4.53	4.04	3.00	1.91	.82	4.99	0
D6S457	3.04	2.98	2.73	2.41	1.73	1.04	.40	3.04	0
D6S1656	−∞	1.07	1.74	1.85	1.58	1.06	.44	1.85	.10

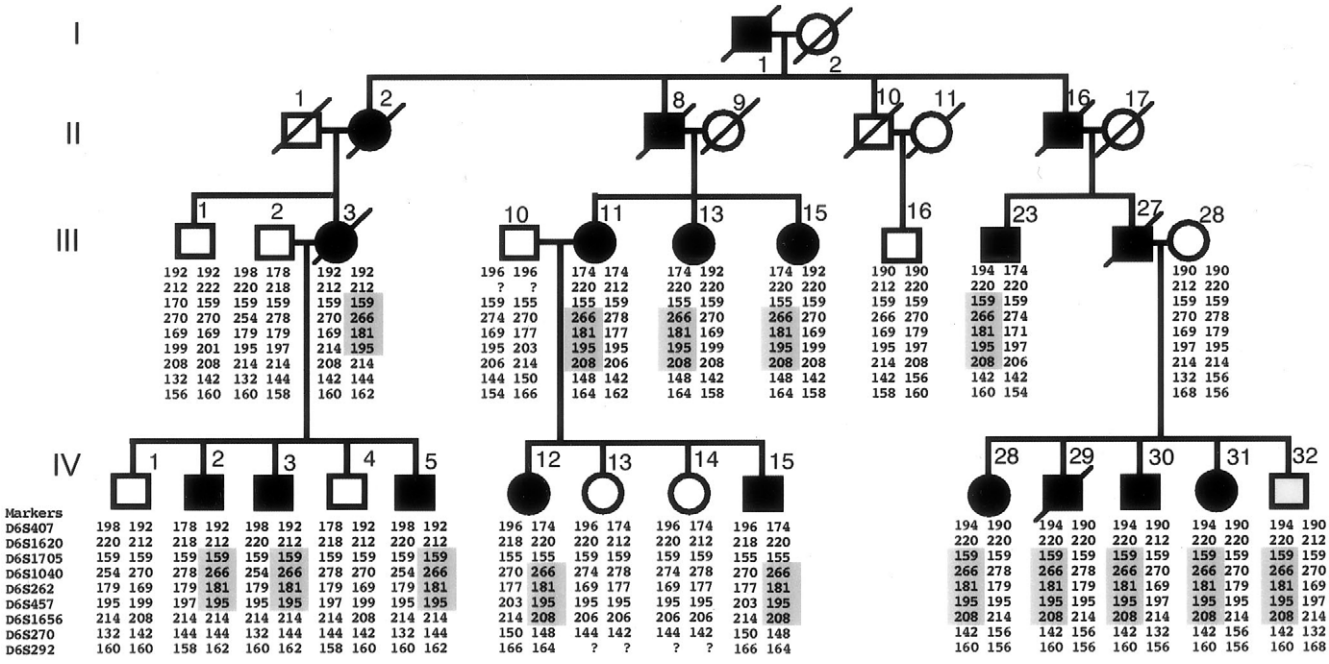

Figure 2 Haplotype analysis of 6q markers in FDC-CDM pedigree. Shown is the FDC-CDM–pedigree subset used for linkage analysis. The numbers and symbols correspond to those shown in figure 1. The markers shown are in the following order: centromere-D6S407, D6S1620, D6S1705, D6S1040, D6S262, D6S457, D6S1656, D6S270, D6S292-telomere. The interval containing the FDC-CDM gene is from D6S1705 to D6S1656.

from D6S1705 to D6S1656 (fig. 3). According to the most recent Généthon map, this interval is 3 cM. Physical-map information from the Whitehead Contig WC6.15 (November 1996) shows a high density of YACs covering the region and that markers defining the FDC-CDM critical interval are contained within a minimum of two YACs with an estimated size of 2.5–3.0 Mb. Preliminary analysis maps a number of expressed sequence tags near or within this interval (data not shown).

Discussion

In this report, we have identified, on the long arm of chromosome 6, a locus that is significantly linked with the development of cardiomyopathy with conduction system disease and LGMD. The phenotype in this disorder, FDC-CDM, is strikingly similar to that described for LGMD type 1B, a disorder that links to the centromere of chromosome 1 (van der Kooi et al. 1996, 1997), demonstrating genetic heterogeneity of autosomal dominant cardiomyopathy and muscular dystrophy. Since LGMD type 1B may be allelic with FDC-CD1, a disease that shows only the cardiac symptoms, it is possible that additional FDC-CDM families may exhibit a pure cardiac phenotype as well. Interestingly, early in their clinical course, several members of the FDC-CDM pedigree had only cardiac features of the disease, and one suffered sudden death prior to developing any symptoms

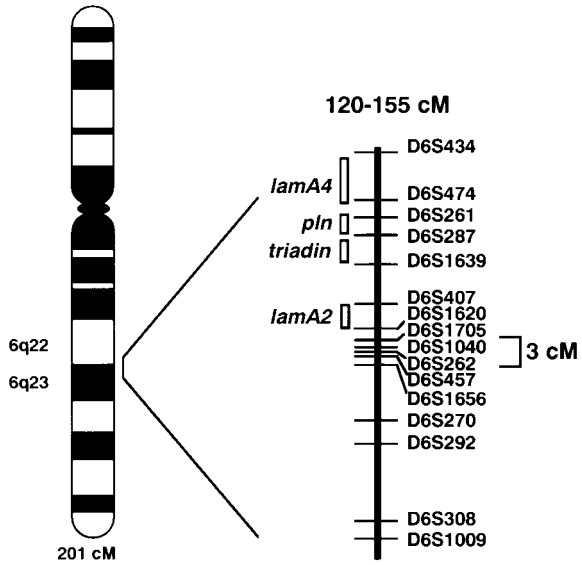

Figure 3 Ideogram of chromosome 6. The markers used in this study derive from the long arm of chromosome 6. The region of 120–155 cM, as based on the March 1996 Généthon map, is shown. Two-point and multipoint linkage analysis identified the markers D6S1040, D6S262, and D6S457 as being significantly associated with the development of autosomal dominant FDC-CDM. Haplotype analysis showing recombination events occurring at D6S1705, D6S1620, and D6S407 excluded the genes centromeric to this region—laminin α2, laminin α4, phospholamban, and triadin. Recombination events were also seen with D6S270, D6S1656, and D6S292, excluding regions telomeric to D6S1656. The other markers listed here were also tested and were consistent with the recombination events described in the text. Therefore, the critical region for FDC-CDM is flanked by D6S1705 and D6S1656. According to the most recent Généthon map, this interval is 3 cM (137–140 cM).

of CHF or muscle weakness. Even within a single family, the phenotype varies, spanning the spectrum of cardiomyopathic and dystrophic symptoms. The association of cardiac conduction-system disease with dilated cardiomyopathy is now seen in three inherited forms of dilated cardiomyopathy (FDC-CD1, FDC-CD2, and FDC-CDM) (Graber et al. 1986; Greenlee et al. 1986). This degree of conduction-system disease is not characteristic of all idiopathic dilated cardiomyopathy patients and may suggest that patients with dilated cardiomyopathy and heart block should be more closely examined for familial disease.

The proximity of the laminin α2 gene (*LAMA2*) to this interval, as well as its involvement in autosomal recessive congenital muscular dystrophy, suggested the possible involvement of laminin α2 mutations in FDC-CDM, an autosomal dominant disease. Mutations in laminin α2 (formerly known as "merosin") have been described in autosomal recessive congenital muscular dystrophy (CMD; OMIM number 156225 [Helbling-Leclerc et al. 1995*a;* Nissen et al. 1996]). CMD patients are hypotonic at birth, and those that are laminin α2 deficient have a particularly poor prognosis, often never achieving independent ambulation (for review, see Bönnemann et al. 1996). T2-weighted cranial magnetic-resonance imaging of laminin α2–deficient CMD patients showed a high degree of white-matter abnormalities, consistent with the expression of laminin α2 in peripheral nerve, brain, and striated muscle (Vainzof et al. 1995). The lack of CNS involvement in the FDC-CDM kindred and the lack of cardiac involvement in the *dy* mouse, a murine model of CMD (Michelson et al. 1955; Meier and Southard 1970), suggest that these are genetically different disorders. The exclusion of laminin α2 as the genetic defect in FDC-CDM is further supported by independent confirmation of the *LAMA2* genetic interval, by two different groups (Helbling-Leclerc et al. 1995*b;* Naom et al. 1997). The most recent data place the *LAMA2* gene centromeric to D6S1620 (Naom et al. 1997). Within the FDC-CDM pedigree, there are recombinants for D6S1620 and D6S1705, excluding the involvement of the *LAMA2* locus in FDC-CDM. To confirm these data, we performed radiation hybrid mapping and independently confirmed the placement of the *LAMA2* gene centromeric to D6S1620 (data not shown). Laminin α4, triadin, and phospholamban also map centromeric to D6S1705 and, therefore, are excluded as being the defective gene in FDC-CDM (Schuler et al. 1996). However, the presence of two laminin genes in this region may suggest a third, since laminin genes can occur in clusters (for review, see Wewer and Engvall 1996). Curiously, laminin genes have also been mapped in 3p22 and 1q32, two regions implicated in inherited cardiomyopathy (Durand et al. 1995; Olson and Keating 1996).

The genetic heterogeneity that now appears to underlie FDC may represent multiple different pathogenetic forms. However, the identification of the defective genes in these disorders may demonstrate that these apparently heterogeneous diseases share similar biochemical pathways. The familial hypertrophic cardiomyopathies (FHC) initially appeared to be genetically diverse (Watkins et al. 1995). It is now known that the multiple loci associated with FHC encode sarcomeric proteins that interact to form the functional unit of contraction. Similarly, the muscular dystrophies initially were thought to be genetically heterogeneous. Mutation analysis has shown that these genetically diverse myopathic disorders arise from defects in genes encoding components of the dystrophin-glycoprotein complex (for reviews, see Campbell 1995; Ozawa et al. 1995; Bönnemann et al. 1996). Genetic analyses of patients and their families with the myopathic disorders have led to a better understanding of the hypertrophic cardiomyopathies and the muscular dystrophies, by permitting phenotype:geno-

type correlations and providing a limited ability to predict prognosis based on mutation data. Furthermore, the identification of genes involved in these disorders has allowed mutation analysis of apparently sporadic forms of the myopathic diseases. Continued studies of genetic causes of FDC should increase our understanding of the myopathic process and should have an impact on therapy for heart failure and muscle dysfunction.

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